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Dear Sir:

DECLARATION UNDER RULE § 1.131

I, Richard A. Anderson, hereby declare that:

1. I am a co-inventor in U.S. Patent Application Serial No. 10/606,038 filed June 25, 2003 and am most familiar with the subject matter of this application and the research effort which

lead to the discovery of the instant invention. All the work described in the following paragraph occurred at the University of Wisconsin located in Madison, Wisconsin, USA.

2. I have reviewed Caenepeel (US 2005/0125852 A1). This reference is not relevant to the above-referenced application. My invention relates to the discovery that the phosphatidylinositol phosphate kinase γ661 isoform (PIPKIγ661) is specifically targeted to focal adhesions by an association with talin. We found that PIPKIγ661 was tyrosine phosphorylated by focal adhesion-associated kinase signaling, increasing both the activity of phosphatidylinositol phosphate kinase and its association with talin. This is in contrast to the teachings of Caenepeel, wherein mammalian protein kinases and protein kinase-like enzymes are described. Phosphatidylinositol phosphate kinases and protein kinases have different and distinct substrates, *i.e.*, lipids versus proteins, and are therefore different enzymes.

3. In any event, as evidenced by the publication entitled "Type 1γ Phosphatidylinositol Phosphate Kinase Targets and Regulates Focal Adhesions" ((November 2002) *Nature* 420:89-93) attached hereto, experiments were being carried out in my laboratory prior to May 9, 2003, which led to the assay disclosed in the above-referenced application. For example, we demonstrated binding of talin with PIPKIγ661 in the presence of an antibody during immunoprecipitation experiments (see page 90, third full paragraph of column 1). While the antibody employed did not appear to modulate binding of talin to PIPKIγ661, this discovery showed

that we could readily detect agents which modulate the interaction between talin and PIPKIγ661.

4. The experiments presented in the above-referenced publication occurred prior to May 9, 2003, as evidenced by a reception date of June 2, 2002 for the manuscript by the journal *Nature*.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

2/11/06
Date

Richard A. Anderson

(see Supplementary Information). Full-length talin 2 clones were PCR-amplified from human brain and skeletal muscle Marathon-Ready cDNA libraries (BD Biosciences Clontech).

Antibodies and constructs

A rabbit serum specific for PtdInsPKIγ-90 was raised against a peptide corresponding to the 28aa-tail (amino acids 635–662 of mouse PtdInsPKIγ-90). The following antibodies were from commercial sources: anti-talin clone 8d4 (Sigma), antiserum C-20 (Santa Cruz Biotechnology) and clone TA205 (Chemicon); anti-vinculin (Sigma) and anti-FAK C-20 (Santa Cruz Biotechnology). Other antibodies used in this study are described elsewhere[6]. The C-terminal domains of human PtdInsPKIγ-87 (amino acids 459–640) and PtdInsPKIγ-90 (amino acids 458–668) were PCR-amplified from clone KIAA0589 and subcloned into pGEX-6P-1 vector (Amersham-Pharmacia). Deletion constructs were generated in the same vector. For expression of GFP fusion proteins, human PtdInsPKIγ-87, -90, 28aa-tail (amino acids 641–668) and PtdInsPKIγ-90 C-terminal (amino acids 384–668) were subcloned into the pEGFPC2 vector (BD Biosciences Clontech). The head of human talin 2 and mouse talin 1, together with fragments from the FERM domain of talin 2, were subcloned into pGex4T1. Recombinant PtdInsPKIγ-90 was produced in insect cells using the baculovirus system (BD PharMingen).

Biochemical procedures

Affinity chromatography experiments and immunoprecipitations were performed from rat brain detergent extract as described previously[6,29]. For the lipid kinase assay, immunoprecipitates were incubated for 10 min at 37 °C in 40 μl kinase buffer (25 mM HEPES pH 7.4, 100 mM KCl, 1 mM EGTA, 2.5 mM $MgCl_2$) containing 20 μg phosphoinositides (Sigma), 20 μCi [γ-^{32}P]ATP and 50 μM cold ATP. Samples were then processed as previously described[6]. For kinase assays involving recombinant PtdInsPKIγ-90, 25 ng enzyme was used. Kinase assays performed with fibroblasts used 50 μg extract. The blot overlay assay with GST and GST–28aa-tail was performed as described[29]. The phage display library screening was performed according to ref. 30.

Cell transfection and immunofluorescence

NIH 3T3 cells were grown and maintained on plastic culture plates at 37 °C in a 5% CO_2 atmosphere in DMEM containing 10% fetal bovine serum. Cells were detached with PBS supplemented with 0.05% trypsin, 1 mM EDTA, replated on fibronectin-coated glass coverslips and transfected using LipofectAmine reagent (Life Technologies). Twenty-four hours later, cells were fixed, immunostained and analysed by either confocal or epifluorescence microscopy. For the quantification of spread cells, 150–200 cells were scored from each of three independent experiments.

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Supplementary Information accompanies the paper on *Nature*'s website (http://www.nature.com/nature).

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Competing interests statement The authors declare that they have no competing financial interests.

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Type Iγ phosphatidylinositol phosphate kinase targets and regulates focal adhesions

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The ability of cells to form cell contacts, adhere to the extracellular matrix, change morphology, and migrate is essential for development, wound healing, metastasis, cell survival and the immune response. These events depend on the binding of integrin to the extracellular matrix, and assembly of focal adhesions, which are complexes comprising scaffolding and signalling proteins organized by adhesion to the extracellular matrix[1–3]. Phosphatidylinositol-4,5-bisphosphate ($PtdIns(4,5)P_2$) regulates interactions between these proteins, including the interaction of vinculin with actin and talin[4–9]. The binding of talin to β-integrin is strengthened by $PtdIns(4,5)P_2$, suggesting that the basis of focal adhesion assembly is regulated by this lipid mediator[9,10]. Here we show that the type I phosphatidylinositol phosphate kinase isoform γ 661 (PIPKIγ661), an enzyme that makes $PtdIns(4,5)P_2$, is targeted to focal adhesions by an association with talin. PIPKIγ661 is tyrosine phosphorylated by focal adhesion associated kinase signalling, increasing both the activity of phosphatidylinositol phosphate kinase and its

association with talin. This defines a mechanism for spatial generation of PtdIns(4,5)P_2 at focal adhesions.

Adherence to extracellular matrix stimulates the generation of PtdIns(4,5)P_2 (refs 4, 6) and potentially modulates focal adhesion assembly around clusters of integrins[1–10]. Type I phosphatidylinositol phosphate kinase isoforms (PIPKIs) generate the lipid messenger PtdIns(4,5)P_2 (refs 11, 12). The PIPKIγ transcripts are alternatively messenger RNA spliced, resulting in the PIPKIγ635 and PIPKIγ661 isoforms that differ by a 26-amino-acid carboxy-terminal extension[13].

PIPKIγ661 is specifically targeted to focal adhesions (Fig. 1a). To determine if the C-terminal 26 amino acids are sufficient for targeting, these residues were fused to green fluorescent protein, GFP. The GFP-γ26 is poorly targeted to focal adhesions, suggesting that other regions within PIPKIγ661 are also required. To define these constraints, we prepared chimaeras using PIPKIα, an enzyme not targeted to focal adhesions (see Supplementary Information). The PIPKIα/γ chimaeras contained 178 residues from the C terminus of PIPKIγ661 (PIPKIα/γ661) or simply the PIPKIγ661 26 amino acids fused to the C terminus of PIPKIα (PIPKIα/γ26). PIPKIα/γ661 and PIPKIα/γ26 target to focal adhesions, but not PIPKIα/γ635 (Fig. 1a). Enhanced targeting of the PIPKIα constructs with the C-terminal 26 residues reflects structural or functional features of the phosphatidylinositol phosphate kinases, such as dimer formation, which is required for activity at membranes[12,14].

We used two approaches to identify proteins that specifically interact with PIPKIγ661. Haemagglutinin (HA)-tagged PIPKIγ661 or PIPKIγ635 were expressed in HEK 293T cells, and isolated by immunoprecipitation. As numerous focal adhesion proteins are tyrosine phosphorylated, the immunoprecipitates were immunoblotted with anti-phosphotyrosine antibodies. A tyrosine-phosphorylated protein of relative molecular mass 230,000 (M_r 230K) was selectively immunoprecipitated with PIPKIγ661 (Supplementary Information). This protein was identified as talin[15,16] (Fig. 1b). Vinculin, focal adhesion associated kinase (FAK) and tensin did not interact with PIPKIγ661 (Supplementary Information). Only the PIPKIα/γ chimaeras that target to focal adhesions associated with talin (Fig. 1b). To confirm the talin–PIPKIγ661 interaction, talin was immunoprecipitated from HEK 293T cells overexpressing PIPKIγ661 or PIPKIγ635. Talin pulled down PIPKIγ661, but not PIPKIγ635 (Fig. 1b). In addition, endogenous PIPKIγ splice isoforms are widely expressed in various cell lines. When endogenous PIPKIγ was immunoprecipitated from a variety of cells types, talin association was observed (Supplementary Information).

A yeast two-hybrid screen using the 178-residue C-terminal region of PIPKIγ661 as bait resulted in the isolation of 12 talin clones. This demonstrated a direct interaction, and narrowed the interacting region to residues 150 to 480 in the talin head region. This region encompasses a portion of F1 and the F2 and F3 regions of talin's 'band 4.1, ezrin, radixin, moesin' (FERM) homology domain. The talin FERM domain also contains regions shown to interact with other focal adhesion proteins[17–20] (Fig. 1c).

These data show that the PIPKIγ661–talin association is important for targeting PIPKIγ661 to focal adhesions. This is reinforced by the immunofluorescent colocalization of PIPKIγ661 and talin in NRK cells (Fig. 2). As PtdIns(4,5)P_2 modulates the association of talin with β-integrin and other focal adhesion proteins[7,9,10], the PIPKIγ661–talin interaction may regulate the focal adhesion assembly of talin. Moderate overexpression of PIPKIγ661 resulted in substantially larger talin-containing focal adhesions in 43% of cells compared to non-transfected cells (Fig. 2). This supports a model where PIPKIγ661 modulates talin assembly into focal adhesions. High overexpression of PIPKIγ661, but not PIPKIγ635, resulted in a loss of talin from focal adhesions in 49% of the transfected cells (Fig. 2). These data indicate that increasing levels of PtdIns(4,5)P_2 at focal adhesions cause talin assembly and disassembly, suggesting a dynamic model. Overexpression of PIPKI isoforms reorganizes actin from stress fibres to cortical actin filaments[11,13]. Thus, phenotypes observed by high overexpression of PIPKIγ661 may also affect focal adhesions indirectly.

Kinase inactive mutants, when expressed in cells, will often disrupt the function of the wild-type kinase by competing for

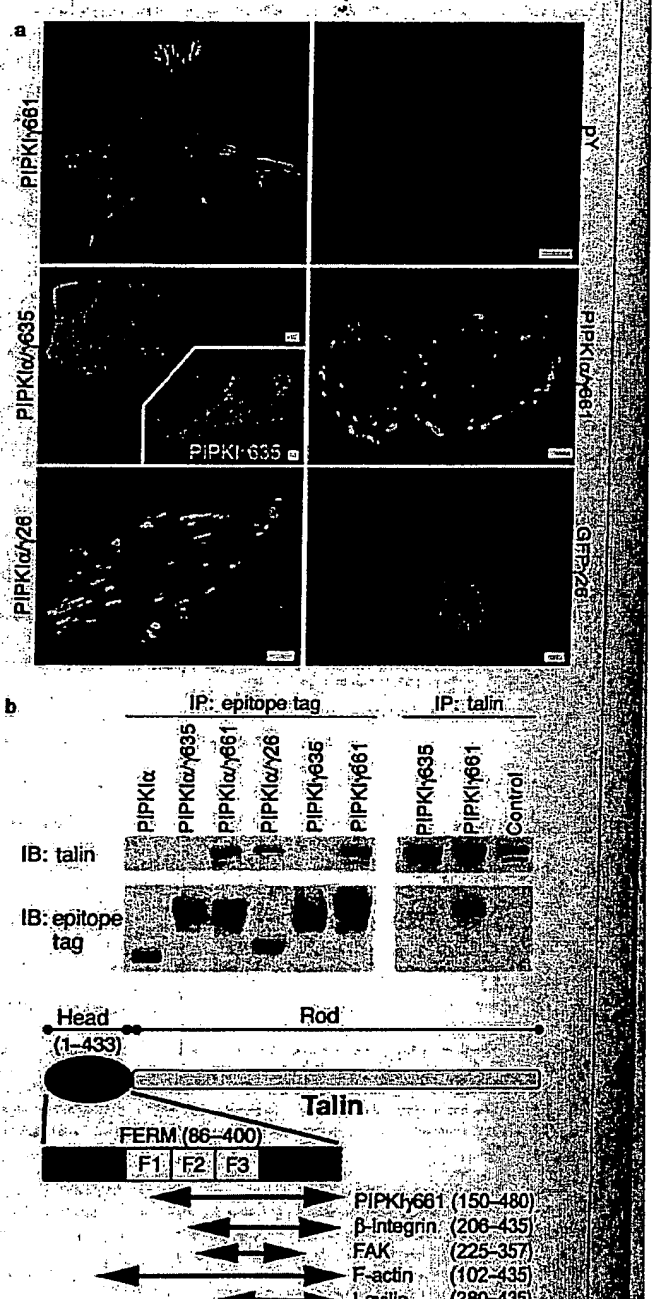

Figure 1 PIPKIγ661 is targeted to focal adhesions by an association with talin. a, Top, colocalization of HA-PIPKIγ661 and focal adhesions in NRK cells stained with anti-HA (green) and polyclonal anti-phosphotyrosine (pY) (red) antibodies. Fusion proteins containing the C-terminal 26 amino acids from PIPKIγ661 show targeting to focal adhesions in NRK cells. c-Myc-PIPKIα/γ chimaeras and HA-PIPKIγ635 were stained with c-Myc and HA antibodies, respectively. See Supplementary Information for generation of chimaeras. Scale bar, 10 μm. b, Overexpressed PIPKI constructs were immunoprecipitated (IP) from HEK 293T cells and then immunoblotted (IB) with indicated antibodies. c, PIPKIγ661 interacts with talin in the FERM domain as determined by yeast two-hybrid screen. Illustration of other talin binding proteins in FERM domain.

targeting or regulation. Expression of a kinase-inactive ('kinase-dead') PIPKIγ661 mutant (PIPKIγ661kd) resulted in a loss of talin targeting to focal adhesions in 70% of the cells expressing high levels (Fig. 2). This is consistent with the proposed role for PtdIns(4,5)P_2 in talin assembly into focal adhesions[1–9].

During adhesion and spreading, cells assemble focal adhesions rapidly, providing a good model to define the role of PIPKIγ661 in membrane and focal adhesion assembly of talin. PIPKIγ661 facilitated the targeting of talin to the plasma membrane in adhering cells (Fig. 3). This was independent of PIP kinase activity, as PIPKIγ661kd also facilitated targeting (Fig. 3 inset; see also Supplementary Information). Significantly, PIPKIγ661 facilitated the assembly of talin-containing focal adhesions in spreading cells, and this was dependent on PIP kinase activity (Fig. 3). These data show that PIPKIγ661 regulates both plasma membrane targeting and the efficient assembly of talin into focal adhesions.

The association between PIPKIγ661 and talin was also regulated by adhesion to type I collagen. In Fig. 4a, we show co-immunoprecipitation of talin with PIPKIγ661 from HEK 293T cells in suspension versus cells adherent to type I collagen. Talin strongly associated with PIPKIγ661 in adherent cells, but not in suspended cells. However, in suspended cells the PIPKIγ661 association with talin was restored when cells were stimulated with lysophosphatidic acid (LPA) or platelet-derived growth factor (PDGF).

Tyrosine phosphorylation of focal adhesion proteins is stimulated by adhesion, and is important for focal adhesion assembly[21]. PIPKIγ661 is tyrosine phosphorylated, and this was dependent upon PIP kinase activity (Fig. 4a). Tyrosine phosphorylation was also stimulated upon cell adhesion to type I collagen. FAK is an important tyrosine kinase at focal adhesions and is activated by LPA[22]. So to determine FAK's role in tyrosine phosphorylation of PIPKIγ661, we coexpressed dominant active FAK (CD2FAK)[23] with each of the PIPKIγ isoforms. This induced a ~4-fold increase in tyrosine phosphorylation of PIPKIγ661, but not PIPKIγ635, illustrating that focal adhesion targeting is required for tyrosine phosphorylation of PIPKIγ661. Tyrosine phosphorylation of PIPKIγ661kd was also stimulated by coexpression of CD2FAK, although to a lesser extent. Coexpression of the dominant-negative FAK related non-kinase (FRNK), which blocks endogenous FAK activity, diminished tyrosine phosphorylation of PIPKIγ661. FRNK also inhibited adhesion-stimulated PIPKIγ661 tyrosine phosphorylation. The FAK-induced tyrosine phosphorylation of PIPKIγ661 correlates with a substantial increase in its association with talin. Tyrosine phosphorylation of PIPKIγ661 may facilitate association with talin via the phosphotyrosine-binding site in the talin head[20]. Significantly, CD2FAK but not FRNK specifically stimulated the activity of PIPKIγ661. These data indicate that FAK signalling stimulates both talin association and PIP kinase activity, resulting in localized generation of PtdIns(4,5)P_2 at focal adhesions (see Supplementary Information).

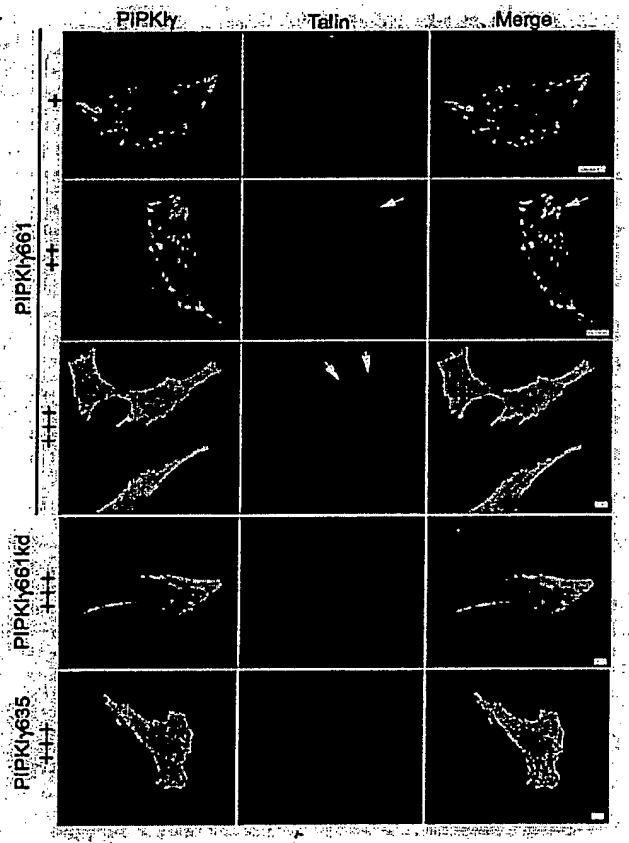

Figure 2 PIPKIγ661 modulates assembly of talin into focal adhesions. Overexpressed PIPKIγ and endogenous talin in NRK cells were visualized by anti-PIPKIγ serum (green) and anti-talin antibody (red), respectively. Relative expression level of PIPKIγ is shown (+). Low HA–PIPKIγ661 (+) colocalized with talin, and the increasing moderate expression (++) induced larger talin-containing focal adhesions, whereas high overexpression (+++) diminished talin assembly into focal adhesions. High expression of PIPKIγ661kd abolished talin assembly into focal adhesions. High expression of PIPKIγ635 constructs had no effect on focal adhesions. Scale bar, 10 μm.

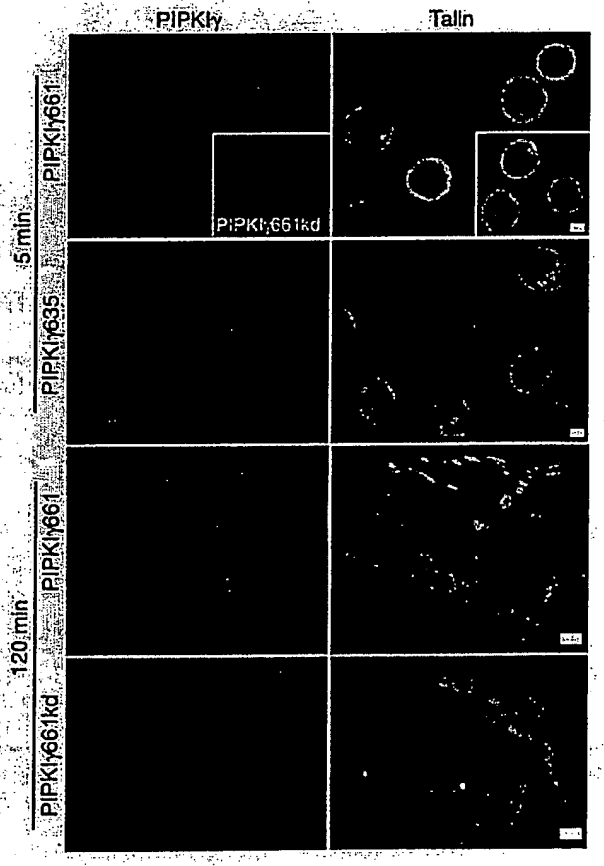

Figure 3 PIPKIγ661 facilitates talin targeting to membranes and assembly into focal adhesions. Transfected NRK cells were suspended for 1 h, then replated on type I collagen (see Methods). After the indicated time, localization of PIPKIγ (green) and talin (red) in cells was visualized with anti-PIPKIγ serum and anti-talin antibodies, respectively. Inset, location of PIPKIγ661kd and talin. See Supplementary Information for quantification of PIPKIγ and talin localization. Scale bar, 10 μm.

A functional link between PIPKIγ661 and FAK was reinforced by the observation that PIPKIγ661kd but not PIPKIγ661 disrupts targeting of FAK to focal adhesions. PIPKIγ661 and PIPKIγ661kd both target to focal adhesions and colocalize with FAK (Fig. 4b and data not shown). High expression of PIPKIγ661 did not affect FAK targeting. In contrast, the expression of PIPKIγ661kd resulted in complete loss of FAK targeting to focal adhesions in 39% of the expressing cells. A number of other focal adhesion proteins were analysed to determine the effect of PIPKIγ661 and PIPKIγ661kd expression. From these studies, paxillin and vinculin were more resistant to changes induced by ectopic expression of PIPKIγ661 or PIPKIγ661kd (see Supplementary Information).

The interaction between PIPKIγ661 and talin appears critical for focal adhesion assembly. This is supported by observations that a β-integrin binding site regulated by PtdIns(4,5)P_2 is located in the head region of talin within the FERM domain[9,17–19]. Proteins containing the FERM domain, such as protein 4.1 and talin, interact with integral membrane proteins, such as glycophorin or integrins, and these interactions are modulated by PtdIns(4,5)P_2 (refs 9, 19, 24, 25). Therefore, the PIPKIγ661–talin interaction may be important in the initiation of focal adhesion assembly via regulation of integrin binding and other focal adhesion proteins by PtdIns(4,5)P_2 (refs 1–3, 7–9).

We suggest that, upon clustering of integrins, talin and PIPKIγ661 are recruited to focal adhesions, inducing synthesis of PtdIns(4,5)P_2. Spatial generation of PtdIns(4,5)P_2 facilitates the recruitment and regulation of proteins such as vinculin, α-actinin and FAK. FAK, in a manner that depends on PtdIns(4,5)P_2, tyrosine phosphorylates PIPKIγ661, and further stimulates production of PtdIns(4,5)P_2. Regulated and localized generation of PtdIns(4,5)P_2 facilitates the assembly and/or disassembly of focal adhesions. PIPKI activity is also regulated by small G proteins[4,6,26] and by phosphatidic acid, a product of phospholipase D (PLD)[27]. PLD activity is required for actin stress fibre formation and α-actinin assembly at focal adhesions[28]. In many cells, adhesion is essential for survival and growth, which require phosphoinositol 3-kinase (PI3K) activity[21]. PI3K is also modulated by FAK, and may be dependent upon PIPKIγ661 for its substrate. Consequently, generation of PtdIns(4,5)P_2 by PIPKIγ661 may also control the generation of messengers derived from PtdIns(4,5)P_2. This positions PIPKIγ661 at a signalling branch point that modulates both focal adhesion assembly and signals emanating from focal adhesions. □

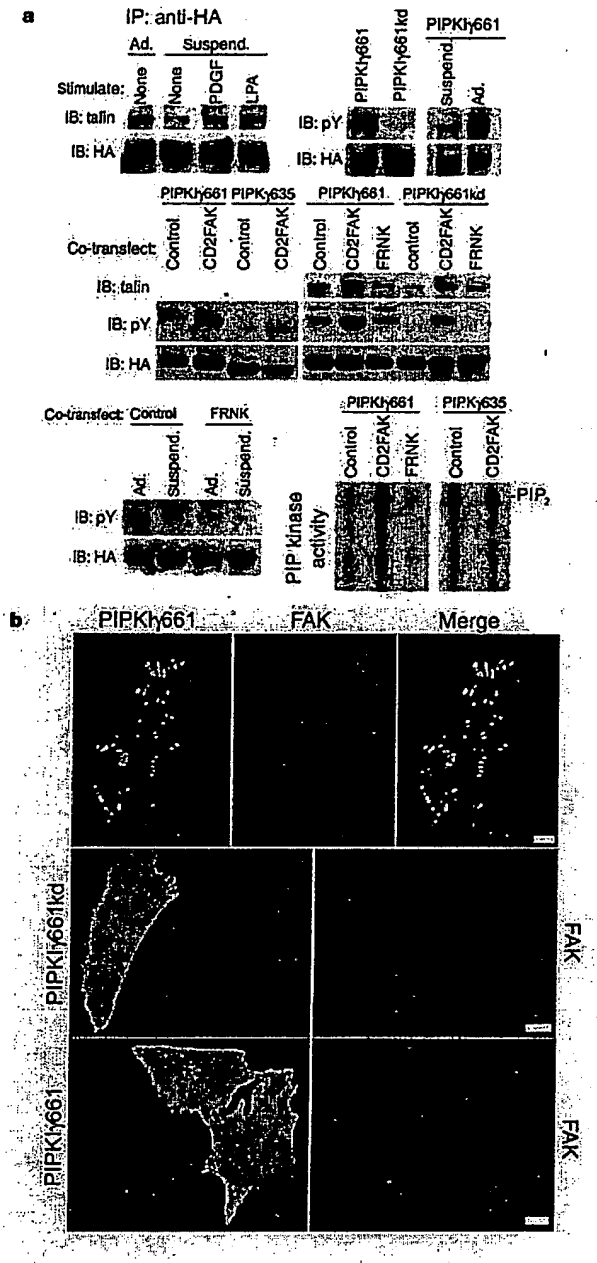

Figure 4 Functional linkage between PIPKIγ661 and FAK signalling. **a**, In all experiments, HA-PIPKIγ was immunoprecipitated (IP) from HEK 293T cells with anti-HA antibody and analysed by immunoblotting (IB) or kinase assay as indicated. Top left, PIPKIγ661–talin association in adherent (ad.), suspended (suspend.), or suspended cells stimulated as indicated. Top right, PIPKIγ661, but not PIPKIγ661kd, is tyrosine phosphorylated, which is dependent upon adhesion. Middle, phosphorylation of PIPKIγ and talin association were shown when cotransfected with CD2FAK or FRNK. Bottom left, adhesion-dependent PIPKIγ661 phosphorylation was inhibited by FRNK. Bottom right, PIPKIγ activity when coexpressed with indicated proteins. (See Supplementary Information for quantification.) **b**, High expression of PIPKIγ661kd but not PIPKIγ661 (visualized by anti-PIPKIγ serum, green) abolishes FAK (visualized with anti-FAK, red) from focal adhesions in NRK cells. Scale bar, 10 μm. $N \geq 4$ for all results in this Letter.

Methods

Constructs

Site-directed mutagenesis for creation of kinase-dead PIPKIγ661 (D253A) was performed using PCR-primer overlap extension with mutagenic primers[12]. The mutagenic primers were 5′-CAAAATGCACCTTAAGTT CGCCCTCAAGGGCTCCAC-3′ and 5′-GTACGTG GAGCCCTTGAGGGCGAA CTTAACCTGC-3′. The flanking primers were 5′-CTATG CACCTGTTGCCTTCCGCTACTTC-3′ and 5′-GGCCGTGGAATACAGAGCCTTC-3′. The mutation was confirmed by DNA sequence analysis. A 0.5-kilobase *Xmal*–*Cla*I restriction fragment was used to replace the corresponding restriction fragment in the HA–PIPKIγ661/pcDNA3 construct. See Supplementary Information for generation of GFP constructs, c-Myc–PIPKIα/γ chimaeras, and recombinant protein expression vectors.

Yeast two-hybrid screen

The yeast two-hybrid screen[29] was performed in the Molecular Interaction Facility at the University of Wisconsin according to their protocols. Libraries screened were: mouse embryonic, human B cell, human breast, human prostate, human placenta and mouse brain. See http://www.biotech.wisc.edu/mif/Lipid

Kinase assays

Activity of purified recombinant or immunoprecipitated PIPKI proteins was assayed against 25 μM PI4P micelles as previously described[12].

Cell culture, transfection, immunofluorescence and confocal microscopy

HEK 293T cells and NRK cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Mediatech, Inc.) supplemented with 10% fetal bovine serum (Invitrogen) and antibiotics. NRK cells were transfected using FuGENE 6 (Roche) according to manufacturer's instructions for 24 h. HEK 293T cells in 1 μg ml^{-1} collagen-coated dishes were transfected using 2 μg PIP kinase expression vector together with 3 μg empty pcDNA3 vector, CD2FAK, or FRNK as indicated, using the calcium phosphate–DNA coprecipitation method for 48 h.

Immunofluorescence and confocal microscopy were performed as described[11]. Z series were created by sequentially scanning green and red channels at 0.2 μm steps. Single sections were exported to Adobe Photoshop 6.0 for final image processing.

Expression and purification of PIP kinase in *Escherichia coli*

His-tagged PIPKIα, PIPKIγ661 and C-tail of PIPKIγ661 were expressed in *E. coli* and purified as described[17].

Antibodies

Anti-talin, anti-vinculin and anti-Flag (M2) antibodies are from Sigma. Monoclonal mouse anti-PY (4G10), anti-FAK (4.47) and anti-paxillin (5H11) are from Upstate. Polyclonal rabbit anti-PY antibody is from Transduction Laboratories. Anti-HA and anti-c-Myc antibodies are from Covance. Polyclonal PIPKIγ anti-serum was generated by Covance using purified His-tagged PIPKIγ661. Secondary antibodies are from Jackson Immunoresearch. Anti-serum was purified on an affinity column generated by coupling recombinant C-terminus of PIPKIγ661 to cyanogen bromide-activated Sepharose 4B (Sigma) as described[30].

Immunoprecipitation and immunoblotting

Immunoprecipitation was performed as described[31]. Briefly, transfected HEK 293T cells were harvested and lysed in 50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 0.1% NP-40, 5.0 mM NaF, 2 mM Na_3VO_4, 4 mM $Na_2P_2O_7$, 1 mM EDTA, 0.1 mM EGTA, 1 mM phenylmethyl sulphonyl fluoride (PMSF), 10% glycerol, then centrifuged and incubated with protein A–Sepharose and 5 μg anti-HA, anti-c-Myc, anti-Flag, or anti-talin antibody as indicated at 4 °C overnight. The immunocomplexes were separated by SDS–polyacrylamide gel electrophoresis (SDS–PAGE), and analysed as indicated. For kinase assay, the immunocomplexes were washed by and stored in kinase buffer.

Cell stimulation and spreading

Transfected HEK 293T cells were washed with PBS and detached with 0.25% trypsin at 37 °C. The cells were harvested and washed with PBS, and then suspended in serum-free DMEM at 37 °C for 1 h. After stimulation with 10 ng ml^{-1} platelet-derived growth factor (PDGF) (R&D Systems Inc.) or 1 μM LPA (Sigma) or serum at 37 °C for 15 min, cells were lysed as above for immunoprecipitation. For spreading assays, transfected NRK cells were detached with 1 mM EDTA, washed with PBS, then 0.1×10^6 cells were plated on 1 μg ml^{-1} collagen-coated coverslips in serum-free DMEM. After the indicated time, the coverslips were fixed and stained as described above.

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Nbs1 is essential for DNA repair by homologous recombination in higher vertebrate cells

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Double-strand breaks occur during DNA replication and are also induced by ionizing radiation. There are at least two pathways which can repair such breaks: non-homologous end joining and homologous recombination (HR). Although these pathways are essentially independent of one another, it is possible that the proteins Mre11, Rad50 and Xrs2 are involved in both pathways in *Saccharomyces cerevisiae*[1]. In vertebrate cells, little is known about the exact function of the Mre11–Rad50–Nbs1 complex in the repair of double-strand breaks because Mre11- and Rad50-